Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/011626

International filing date: 06 April 2005 (06.04.2005)

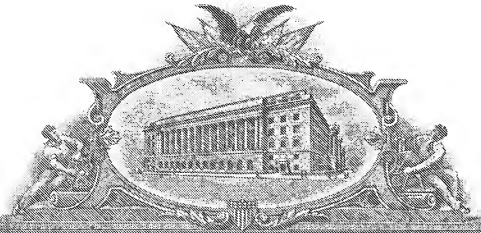
Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/625,871
Filing date: 08 November 2004 (08.11.2004)

Date of receipt at the International Bureau: 12 August 2005 (12.08.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse



THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

August 04, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: ***60/625,871***
FILING DATE: ***November 08, 2004***
RELATED PCT APPLICATION NUMBER: ***PCT/US05/11626***

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. EV 439402995 US

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Joseph R.	Garlich	328 W. Columbine Lane, Westfield, IN 46268
Taxiarchis M.	Georgiadis	

Additional inventors are being named on the 1 *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max):

PTEN INHIBITORS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☑ The address corresponding to Customer Number: 08-3038

OR

☐ Firm or Individual Name

Address

City	State	Zip
Country	Telephone	Fax

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 32

☐ CD(s), Number of CDs

☑ Drawing(s) *Number of Sheets* 24

☑ Other (specify) Transmittal letter, Fee Trasnmittal, and return-receipt postcard

☐ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☑ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees.

FILING FEE Amount ($) 80.00

☐ Payment by credit card. Form PTO-2038 is attached.

☑ The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 08-3038
A duplicative copy of this form is enclosed for fee processing.

☐ The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:

SIGNATURE [signature] Date November 8, 2004

TYPED or PRINTED NAME Teddy C. Scott, Jr., Ph.D. REGISTRATION NO. 53,573 *(if appropriate)*

TELEPHONE (312) 846-5621 Docket Number: 01656.0011.PZUS00

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

16198 U.S. PTO 110804

22964 U.S. 60/625871 110804

PROVISIONAL APPLICATION COVER SHEET
Additional Page

PTO/SB/16 (09-04)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

First Named Inventor	Joseph R. Garlich	Docket Number	01656.0011.PZUS00

INVENTOR(S)/APPLICANT(S)

Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)
Xiaodong	Peng	
Jin	Su	
Tim C.	Smith	

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

PTO/SB/17 (10-04v2)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

FEE TRANSMITTAL for FY 2005

Effective 10/01/2004. Patent fees are subject to annual revision.

☑ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	Not yet assigned
Filing Date	November 8, 2004
First Named Inventor	Joseph R. Garlich
Examiner Name	Not yet assigned
Art Unit	Not yet assigned
Attorney Docket No.	01656.0011.PZUS00

METHOD OF PAYMENT *(check all that apply)*

☐ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 08-3038

Deposit Account Name: Howrey Simon Arnold & White LLP

The Director is authorized to: *(check all that apply)*

☑ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	790	2001	395	Utility filing fee	
1002	350	2002	175	Design filing fee	
1003	550	2003	275	Plant filing fee	
1004	790	2004	395	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

	Extra Claims		Fee from below	Fee Paid
Total Claims		-20** =	x	=
Independent Claims		- 3** =	x	=
Multiple Dependent				=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	88	2201	44	Independent claims in excess of 3
1203	300	2203	150	Multiple dependent claim, if not paid
1204	88	2204	44	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) -0-

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	430	2252	215	Extension for reply within second month	
1253	980	2253	490	Extension for reply within third month	
1254	1,530	2254	765	Extension for reply within fourth month	
1255	2,080	2255	1,040	Extension for reply within fifth month	
1401	340	2401	170	Notice of Appeal	
1402	340	2402	170	Filing a brief in support of an appeal	
1403	300	2403	150	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,370	2453	685	Petition to revive - unintentional	
1501	1,370	2501	685	Utility issue fee (or reissue)	
1502	490	2502	245	Design issue fee	
1503	660	2503	330	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	790	2809	395	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	790	2810	395	For each additional invention to be examined (37 CFR 1.129(b))	
1801	790	2801	395	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid **SUBTOTAL (3)** ($) -0-

SUBMITTED BY				(Complete *(if applicable)*)	
Name (Print/Type)	Teddy C. Scott, Jr., Ph.D.	*Registration No. (Attorney/Agent)*	53,573	*Telephone*	312 846-5621
Signature				*Date*	November 8, 2004

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO:** Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

PTO/SB/21 (09-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Total Number of Pages in This Submission: 60

Application Number	Not yet assigned
Filing Date	November 8, 2004
First Named Inventor	Joseph R. Garlich
Art Unit	Not yet assigned
Examiner Name	Not yet assigned
Attorney Docket Number	01656.0011.PZUS00

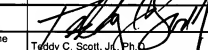
ENCLOSURES *(Check all that apply)*

- [x] Fee Transmittal Form
- [ ] Fee Attached
- [ ] Amendment/Reply
  - [ ] After Final
  - [ ] Affidavits/declaration(s)
- [ ] Extension of Time Request
- [ ] Express Abandonment Request
- [ ] Information Disclosure Statement
- [ ] Certified Copy of Priority Document(s)
- [ ] Reply to Missing Parts/ Incomplete Application
  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53
- [ ] Drawing(s)
- [ ] Licensing-related Papers
- [ ] Petition
- [ ] Petition to Convert to a Provisional Application
- [ ] Power of Attorney, Revocation Change of Correspondence Address
- [ ] Terminal Disclaimer
- [ ] Request for Refund
- [ ] CD, Number of CD(s) _____
  - [ ] Landscape Table on CD
- [ ] After Allowance Communication to TC
- [ ] Appeal Communication to Board of Appeals and Interferences
- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
- [ ] Status Letter
- [x] Other Enclosure(s) (please Identify below):

Provisional Patent Application Cover Sheet (2 pgs.), and return-receipt postcard.

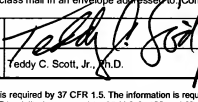
Remarks

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm Name	Howrey Simon Arnold & White, LLP		
Signature			
Printed name	Teddy C. Scott, Jr., Ph.D.		
Date	November 8, 2004	Reg. No.	53,573

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below:

Signature			
Typed or printed name	Teddy C. Scott, Jr., Ph.D.	Date	November 8, 2004

This collection of information is required by 37 CFR 1.5. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and1.14. This collection is estimated to 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: **Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Patent Application: **PTEN Inhibitors**

List of Contributors: Taxiarchis M. Georgiadis, Ph.D.,
Joseph Garlich, Ph.D.,
Xiaodong Peng, Ph.D.,
Jin Su, Ph.D.,
Tim C. Smith, Ph.D.,

Summary

We have developed an assay that measures PTEN activity and have tested compounds in search of potent PTEN inhibitors. To date we have tested approximately 250 compounds and have found some activity in four distinct chemical entities.

Background and Existing Knowledge- PTEN

Cellular processes are to some extent controlled by cycles of phosphorylation and dephosphorylation involving lipids and proteins. PTEN (phosphatase located on chromosome 10) is a dual specificity phosphatase which dephosphorylates an important lipid second messenger, phosphatidylinosital 3,4,5 phosphate [Ptlins(3,4,5)P3] to control cell division and apoptosis. It is mutated at high frequency in human malignant disease (incidence varies from 20% to 95% depending on tumor type). Preliminary data from the Durden group has implicated PTEN in the control of tumor-induced angiogenesis and the control of immunoreceptor signaling suggesting that this is a major drug target for control of angiogenesis and inflammatory signals.

What we hope to achieve

We believe an agent which would inhibit PTEN thereby augmenting levels of PIP3 would likely have therapeutic efficacy in a number of disease states associated with uncontrolled cell death and tissue damage

Therapeutic areas

Chemistry

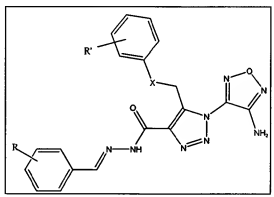
We have screened for compounds that would bind to PTEN and inhibit phosphorylation. PTEN hydrolyzes phosphate at the 3 position on the inositol ring of PtdIns(3,4,5)P3, and Ins(1,3,4,5)P4. The release of phosphate from the natural substrate was measured in a colorimetric assay by using the Malachite Green Reagent (Upstate) in accordance with the instructions of the manufacturer. The absorbance at 650 nm was recorded in an

ELISA plate reader. A standard curve was performed in each assay, and the amount of free phosphate was calculated from the standard curve line-fit data.

From an initial screening of 100 samples selected from insight using an *in silica* interaction of protein to commercially available samples, we were able to identify four initial series (and later a fifth series from literature screening) that shows some preliminary activity at 250uM concentrations. These series were pursued and we identified four distinct series that have been found to show modest activity in the PTEN assay. We have labeled the series as follows: 1) Furazan Series, 2) Diamide Series, 3) Sulf-hydrazone Series, and 4) Peptide Series. In addition, based on these results and searching relevant literature, a fifth series, the Diketone Series was discovered along with a number of other, miscellaneous compounds. All samples reported are represented using our internal numbering system (originally called "CC" numbers but later changed to our current "SF" numbering system) and individual lots are characterized by unique batch code numbers and notebook page numbers.

1.0 ChemNavigator Derived Series

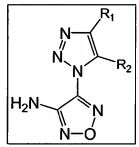
1.1 Furazan Series



Batch Number	Compound Number	Notes	R Group (R'=H)	X Group	% Inhibition at 250uM	% Inhibition at 250uM	IC50 (uM)
BC100108	CC1523-000	2nd batch	3-(OEt),4-(OCH2CONH2)	O	65	52.9	135
BC100041	CC1523-000		3-(OEt),4-(OCH2CONH2)	O	73.6	76.4	
BC100033	CC1515-000		4-Br	N	56		
BC100171	CC1515-000		4-Br	N	38.3	46.2	

BC100143	CC1623-001		3-NO2	O	16	50.1	

The initial hits **CC1523** and **CC1515** were explored. The core ring system was examined and a number of analogs were screened. As shown below, analogs that did not contain a phenyl hydrazide group in the R1 position and instead contained a precursor ethyl ester had little activity at 250uM. Likewise, it was apparent that aryloxy methylene or aryl amino methylene groups in the R2 position were important for activity.



Compound Number	Barcode Number	Notebook Number	R1	R2	%Inhibition at 250uM	IC50 (uM)
CC1507-000	BC100025	A033-48	CONHNCHPh(3Br)	CH2OMe	-14.25	
CC1512-000	BC100030	A033-48	CH3	C(Me)NNH2	6.8	
CC1515-000	BC100033	A033-47	CONHNCHPh(4Br)	CH2NHPh	56, 38.3	
CC1515-000	BC100171	A033-70-22	CONHNCHPh(4Br)	CH2NHPh	46.2	
CC1515-000	BC100186	A033-77-14	CONHNCHPh(4Br)	CH2NHPh	46.2	
CC1521-000	BC100039	A033-47	CONHNCHPh(3-OEt, 4-OCH2Ph(2-Cl,6-F))	CH2NEt2	-11.8	
CC1523-000	BC100108	A033-56-9	CONHNCHPh(3-OEt, 4-OCH2CONH2)	CH2OPh	65, 52.8	245
CC1523-000	BC100041	A033-47	CONHNCHPh(3-OEt, 4-OCH2CONH2)	CH2OPh	73.6,76.4,84.5	135
CC1533-000	BC100051	A033-38-2	CO2Et	CH2NHCHCH(OH)Ph	-20	
CC1541-000	BC100059	A033-38-10	CO2NHNCHPh(3-Br, 4-OH, 5-OMe)	CH2NEt2	-10	
CC1618-000	BC100138	A033-70-17	CONHNHCOPh	Ph	-1.7	

CC1619-000	BC100139	A033-70-18	CO2NHNCHPh(3-Br, 4-OH, 5-OMe)	Ph	18.7	
CC1620-000	BC100140	A033-70-19	CO2NHNCHPh(3,4,5-triOMe)	Ph	18.6	
CC1623-001	BC100143	A033-70-23	CONHNCHPh(3-NO2)	CH2NHPh	50.1	
CC1623-001	BC100187	A033-77-15	CONHNCHPh(3-NO2)	CH2NHPh	45.5	
CC1633-000	BC100153	A033-88-1	CO2Et	CH2NHPh	-11.1	
CC1634-000	BC100154	A033-88-2	CO2Et	CH2OPh	-14.4	
CC1635-000	BC100155	A033-88-3	CO2Et	CH2S-2benzo[d]thiazole	21.2, 15.5	
CC1636-000	BC100156	A033-88-4	CO2Et	CH2-indoline	9.2	
CC1637-000	BC100157	A033-88-5	CO2Et	CH2S-(1-Mel-1H-imidazol)	-11.2	
CC1638-000	BC100158	A033-88-6	CO2Et	CH2N(Me)CH2Ph	-4.1	
CC1639-000	BC100159	A033-88-7	CO2Et	CH2S(5-NH2-1,3,4-thiadiazol-2-yl)	-9.1	
CC1640-000	BC100160	A033-88-8	CO2Et	CH2S(1H-benzo[d]imidazol-2-yl)	2.1	
CC1641-000	BC100161	A033-88-9	CO2Et	CH2(1H-benzo[d][1,2,3]triazol-1-yl)	-7	
CC1642-000	BC100162	A033-88-10	CO2Et	CH2NHCH2(2-Py)	0.8	
CC1643-000	BC100163	A033-88-11	CO2Et	CH2S(4,6-dimethylpyrimidin-2-yl)	0.3	
CC1644-000	BC100164	A033-88-12	CO2Et	CH2NHPh(4-OMe)	12.4,10.5	
CC1645-000	BC100165	A033-88-13	CO2Et	CH2N(CH2CH2)2N(2-Py)	0.9	
CC1726-000	BC100262	A048-41-1	CO2Et	NHCH2Ph(3-Br, 4-Me)	25.4	
CC1728-000	BC100264	A048-41-3	CONHNCHPh(3,4-diOMe)	Me	34	

We plan to continue looking for additional analogs. With respect to the R1 Group, we plan to optimize the spacer unit (length and composition) as well as determine optimal substitution on the terminal aromatic ring and determine if substituted aliphatic rings

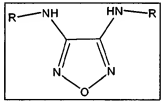
enhance activity). With respect to the R2 Group, we plan to optimize the spacer (currently -CH2O-, -CH2NH-, -NHCH2-, -CH2S-, -CH2-) as well as determine optimal substitution on the terminal aromatic ring (and determine if substituted aliphatic rings enhance activity). We also plan to examine the significance of the amino furazan ring and determine the effect of substituting with other substituted aryl rings.

Diamide Series

The Diamide Series started out as a symmetrical molecule with a core ring system comprised of a furazan ring **(SF 1518)**. Derivatives were synthesized to determine the inhibitory effect of the symmetrical R groups, the core ring system, and the symmetry of the molecules.

1.2.1 R Group Derivatives

1.2.2



From the initial study it appeared that **SF1609** and **SF1617** has an IC50 ~100uM.

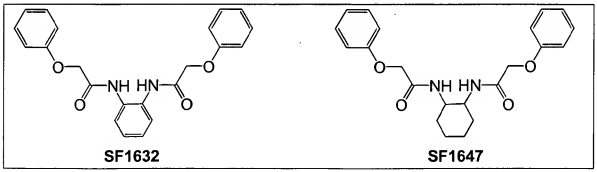
SF Number	Notebook #	Barcode_#	R	%Inh.@250uM	IC50 (uM)
SF1518-000	A033-47	BC100036	COCH2OPh(3-Me)	46.6	
SF1607-000	A033-70-6	BC100127	COCH2OPh(2-OMe)	11.3	
SF1607-000	A033-77-2	BC100174	COCH2OPh(2-OMe)	10.7	
SF1608-000	A033-70-7	BC100128	COCH2OPh(4-Br)	41.8, 26.3	
SF1608-000	A033-77-3	BC100175	COCH2OPh(4-Br)	25.9	
SF1609-000	A033-70-8	BC100129	COCH2OPh(2,5-diMe)	77.7, 76, 79.8	80uM, 113uM
SF1609-000	A033-77-4	BC100176	COCH2OPh(2,5-diMe)	58.4 *	
SF1610-000	A033-70-9	BC100130	COCH2OPh(2-iPr,5-Me)	39.3	
SF1610-000	A033-77-5	BC100177	COCH2OPh(2-iPr,5-Me)	28.3	
SF1611-000	A033-70-10	BC100131	COCH2OPh(4-OMe)	33.8	
SF1611-000	A033-77-6	BC100178	COCH2OPh(4-OMe)	19.7	
SF1612-000	A033-70-11	BC100132	COCH2OPh	82	

SF1612-000	A033-77-7	BC100179	COCH2OPh	3.3	
SF1614-000	A033-70-13	BC100134	COCH2OPh(2-Me)	41.9	
SF1614-000	A033-77-9	BC100181	COCH2OPh(2-Me)	56.5	
SF1615-000	A033-70-14	BC100135	COCH2OPh(4-Me)	38.5	
SF1615-000	A033-77-10	BC100182	COCH2OPh(4-Me)	46.2	
SF1616-000	A033-70-15	BC100136	COCH2OPh(2-iBu)	13.5	
SF1616-000	A033-77-11	BC100183	COCH2OPh(2-iBu)	40.8	
SF1617-000	A048-41-4	BC100265	COCH2OPh(2-iPr)	75.5, -16.3*	84uM
SF1617-000	A048-43-4	BC100270	COCH2OPh(2-iPr)	78.2, 3.4*	
SF1617-000	A033-70-16	BC100137	COCH2OPh(2-iPr)	81.1, 30.2*	66uM
SF1617-000	A033-77-12	BC100184	COCH2OPh(2-iPr)	77.7, 34.7*	
SF1617-000	A048-56-2	BC100283	COCH2OPh(2-iPr)	39.6*	

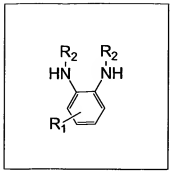
* Repeated using slightly different assay conditions.

1.2.2 Core Group Derivatives-Symmetrical Phenyl

In addition to modifying the terminal R groups we also examined the effect of substituting the furazan ring with other ring systems, both aromatic and aliphatic. We determined that a planar orientation was preferable based on the cyclohexane analog (**SF1647**, 2.2% inhibition@250uM) compared to the phenyl analog (**SF1632**, 31.3% inhibition@250uM). We plan to further examine the effect of 1,2 substitution with 1,3 and 1,4 substitution on the core aromatic ring.



We synthesized derivatives of **SF1647** and have shown the results in the table below. These compounds all have a 1,2 disubstituted aromatic core ring with symmetrical substitution.



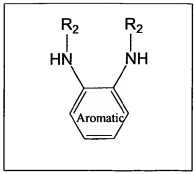
SF Number	Notebook #	Barcode_#	R1	R2	%Inh.@250uM
SF1632-000	A033-77-16	BC100188	H	COCH2OPh	31.3
SF1632-000	A033-67D	BC100152	H	COCH2OPh	31.5
SF1648-000	A048-10-1	BC100229	H	COCH2OPh(2-iPr)	4.9
SF1648-000	A033-84	BC100168	H	COCH2OPh(2-iPr)	-15.5
SF1649-000	A033-93	BC100169	H	COCH2OPh(2-OMe)	-13.2, -22.5
SF1695-000	A033-87-1	BC100218	H	COCH2OPh(4-OMe)	34.4 , 13.2*
SF1695-000	A048-14B	BC100235	H	COCH2OPh(4-OMe)	40.2
SF1701-000	A033-92	BC100223	H	COCH2OPh(2-Me)	51.4, 32.9
SF1703-000	A033-95T	BC100225	H	COCH2OPh(3-OMe)	32.8
SF1712-000	A048-19B	BC100237	H	COCH2OPh(4-OMe)	56.5, 69.3, 15.5*
SF1646-000	A033-76-B	BC100167	4-Me	COCH2OPh	33.7, 34.6, 1.8*
SF1696-000	A033-76-C	BC100219	4-CO2H	COCH2OPh	29.2
SF1697-000	A033-76-D	BC100220	3-Me	COCH2OPh	9.1
SF1713-000	A048-20	BC100238	4-Me	COCH2OPh(4-OMe)	31.5, 66.5, 17.8*
SF1744-000	A048-58-3	BC100286	4CO2H	COCH2OPh(4-OMe)	-18

* Repeated using slightly different assay conditions.

We plan to continue looking for additional analogs. With respect to the R Group, we plan to optimize the spacer unit (length and composition) as well as determine optimal substitution on the terminal aromatic ring (and determine if substituted aliphatic rings enhance activity).

1.2.3 Core Group Derivatives-Symmetrical Aromatic Rings

In addition to phenyl rings, other aromatic rings were incorporated into the core ring system. These include pyridyl and pyrimidyl rings.



SF Number	Notebook #	Barcode_#	Center Ring	R2	%Inh.@240uM
SF1698-000	A033-76-E	BC100221	pyrimidine	COCH2OPh	38.5, 36.1*
SF1747-000	A048-58-6	BC100289	Pyridine#	COCH2OPh(4-OMe)	-33.5

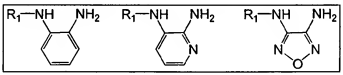
2,3 disubstituted pyridine

* Repeated using slightly different assay conditions.

We plan to examine the effect of replacing the furazan ring with a variety of other heterocycles, including pyridine, pyrimidine, thiophene, furan ring systems.

1.2.4 Core Group Derivatives-Asymmetrical Aromatic Rings

In addition, we examined the symmetrical nature of the series. We synthesized some mono-substituted core rings. Each compound contains only one side group on the aromatic di-amine.



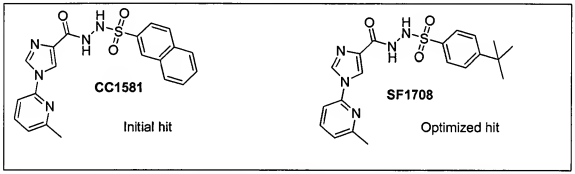
SF Number	Notebook #	Barcode_#	Core Ring	R1	%Inh.@240uM	IC50 (uM)
SF1700-000	A033-96-A	BC100170	benzene	COCH2OPh(2-iPr)	-18.4	
SF1704-000	A033-95B	BC100226	benzene	COCH2OPh(3-OMe)	56.6, (96.1)*	220uM
SF1706-000	A048-10-2	BC100230	benzene	COCH2OPh(2-iPr)	10.6	
SF1710-000	A048-14A	BC100234	benzene	COCH2OPh(4-Me)	4.6	
SF1711-000	A048-19A	BC100236	benzene	COCH2OPh(4-OMe)	-13.6	
SF1742-000	A048-58-1	BC100284	benzene (3-Me)	COCH2OPh(4-OMe)	-21.9	

SF1745-000	A048-58-4	BC100287	benzene(SCO2H)	COCH2OPh(4-OMe)	-25.4	
SF1746-000	A048-58-5	BC100288	pyridine(2-NH2)	COCH2OPh(4-OMe)	-3.5	
SF1748-000	A048-58-7	BC100290	furazan	COCH2OPh(4-OMe)	-11.9	

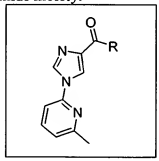
* Repeated using slightly different assay conditions.

We plan to continue looking for additional analogs. With respect to the symmetry, we plan to determine if unsymmetrical analogs display better activity. We plan to examine the role of the primary amine (in the above mentioned cases). Unsymmetrical disubstituted core ring systems are also envisioned.

1.3 Sulf-hydrazone Series



In the Sulf-hydrazone series, our initial hit **CC1581** was derivatized with emphasis on manipulating the terminal aryl amide moiety.



Compound Number	Batch Code Number	R Group	% Inhibition at 250uM	IC50 (uM)
SF1581-000	BC100210	NHNHSO2(2-Naphthyl)	76.6	190
SF1581-000	BC100100	NHNHSO2(2-Naphthyl)	55.6, 41.3, (-3.5)*	
SF1688-000	BC100211	NHNH2	7.1	
SF1689-000	BC100212	NHNHCOPh(4-Me)	6	
SF1690-000	BC100213	NHNHCOPh(4-Br)	22.7	
SF1691-000	BC100214	NHNHSO2Ph(4-OMe)	6.9	

SF1692-000	BC100215	NHNCHPh(4-NO2)	45.5, 38.9, (-1.5)*	
SF1693-000	BC100216	NHNHSO2Ph(3-CF3)	13.6	
SF1694-000	BC100217	NHNCHPh	12.9	
SF1699-000	BC100222	NHNHCO(2-naphthyl)	42.8, 41.4, (-13.4)*	
SF1702-000	BC100224	NHNHSO2Ph(4-Me)	46.4, 43.7, (-10.1)*	
SF1707-000	BC100231	NHNHSO2Ph(4-tBu)	25.1	
SF1708-000	BC100268	NHNHCOPh(4-tBu)	95.5, 84.6	50
SF1708-000	BC100269	NHNHCOPh(4-tBu)	-8.6*	
SF1708-000	BC100232	NHNHCOPh(4-tBu)	56.9*	
SF1709-000	BC100233	NHNHCO(CH2)3)Ph	28.5	
SF1714-000	BC100239	OH	8	
SF1730-000	BC100267	NHNHCOPh(4-NO2)	-9.7	
SF1731-000	BC100271	NHNHSO2Ph(4-NO2)	8.3*	
SF1739-000	BC100280	NHNCHPh(4-tBu)	-14.3*	
SF1775-000	BC100319	OMe	18.3*	

* Repeated using slightly different assay conditions.

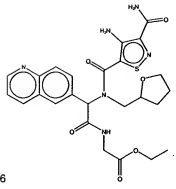
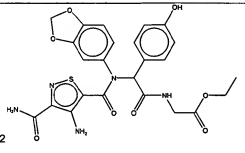
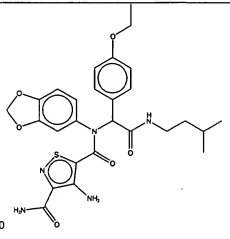
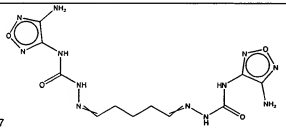
We plan to continue looking for additional analogs. We plan to examine the spacer unit between the biaryl and the terminal aryl ring. In addition we plan to incorporate other biaryl functionalities.

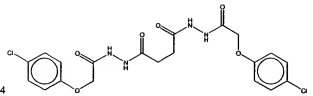
1.4 Peptide Series

1.5

From the initial screening of 100 compounds there were several hits that were peptide like. They consisted of long aliphatic chains with characteristic amide bonds. We have not optimized these series to date.

Structure	Sample Number	Batch Number	Semafore Number	% Inhibition at 250uM

 Structure86	A033-55-12	BC100085	CC1566-000	70.6%
 Structure72	A033-54-10	BC100071	CC1552-000	52.4%
 Structure80	A033-55-6	BC100079	CC1560-000	37.1%
 Structure87	A033-56-1	BC100086	CC1567-000	60.2%

Structure34 	A033-47	BC100034	CC1516-000	35.3

1.5 Summary of the ChemNavigator series

The four series initiated from the ChemNavigator *in silica* database search yielded several hits. For this specific effort, a hit is defined as a compound exhibiting greater then 30% inhibition at 250 uM in the PTEN assay. Our initial optimization of these series produced several micromolar inhibitors in the PTEN assay.

SF#	PTEN	Series
SF1523	135uM	Furazan Series
SF1609	80uM	Diamide Series
SF1617	66uM	Diamide Series
SF1708	50uM	Sulf hydrazone Series

2.0 Semafore Derived Series

Inspired from the work of Urbanek et al (*J.Med Chem* **2001**, *44*, 1777-1793) on their work on potent reversible Inhibitors of Protein Tyrosine Phosphatase CD45 and from our internal research efforts, we examined the PTEN activity of a series of diketone compounds. We hypothesize that the diketone moiety would be capable of reacting with the PTEN active site cystine and that we could engineer in selectivity by taking advantage of PTEN's larger catalytic site compared to other phosphatases. We have identified two series of compounds, labeled Diketone Phananthrolines and Diketone Phenanthrenes, that exhibit greater the 95% inhibition @ 250 uM in the PTEN assay. In addition, several miscellaneous compounds were found to have modest micromolar inhibition in the PTEN assay.

We examined a list of known PTP inhibitors and tested them in the PTEN assay. We discovered that several vanadate compounds (known PTP1B inhibitors) also exhibit PTEN activity. Recently the Woscholski group at Imperial College, England (Schmid *et al., FEBS* **2004**, *566*, 35-38), reported PTEN activity for several oxovanadates (**CC1668,**

CC1664, CC1674, CC1675).

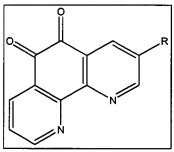
Barcode Number	Sample Number	Compound Name	% Inhibition at 250 uM	IC50 (uM)
BC100189	CC1668-101	Potassium Bisperoxo(bipyridine)oxovanadate (V)	48.1	
BC100190	CC1669-100	Alendronate, Sodium, Trihydrate	-0.9	
BC100191	CC1670-000	N-(9,10-Dioxo-9,10-dihydro-phenanthren-2-yl)-2,2-dimethyl-propionamide	99.7	2
BC100192	CC1671-000	5-Benzyl-3-furylmethyl (1R,S)-cis,trans-chrysanthemate	9.6	
BC100193	CC1672-100	Suramin, Sodium Salt; 8,8'-[carbonylbis[imino-3,1-phenylene carbonyl imino(4-methyl-3, 1-phenylene)carbonylimino]]bis-, hexa sodium salt	-9.9	
BC100194	CC1673-000	4-Methoxyphenacyl Bromide	2	
BC100195	CC1674-101	Dipotassium Bisperoxo(5-hydroxypyridine-2-carboxyl)oxovanadate (V)	60	
BC100196	CC1675-101	Dipotassium Bisperoxo(picolinato)oxovanadate (V)	66.2	
BC100197	CC1676-000	1,4-Dimethylendothall;1,4-Dimethyl-7-oxabicyclo[2.2.1]heptane-2,3-dicarboxylic Acid	3.3	
BC100198	CC1677-000	Monoperoxo(picolinato)oxovanadate(V)	43.9	
BC100199	CC1678-101	Potassium Bisperoxo(1,10-phenanthroline)oxovanadate (V)	92	7
BC100200	CC1679-000	Cantharidic Acid; 2,3-dimethyl-7-oxa-bicyclo[2.2.1]heptane-2,3-di carboxylic acid	2.7	
BC100201	CC1680-000	Sodium Stibogluconate; Antimony Sodium Gluconate	1.5	
BC100202	CC1681-000	3,4-Dephostatin, Ethyl-	82.4	50
BC100203	CC1682-000	bis(N,N-Dimethylhydroxamido)hydroxooxovanadate	26.2	
BC100204	CC1683-000	Fenvalerate; a-Cyano-3-phenoxybenzyl-a-(4-chlorophenyl)isovalerate	-25.5	
BC100205	CC1684-100	α-Naphthyl Acid Phosphate, Monosodium Salt	11.2	
BC100206	CC1685-100	β-Glycerophosphate, Disodium Salt, Pentahydrate	10.9	
BC100207	CC1686-000	Endothall; 7-Oxabicyclo[2.2.1]heptane-2,3-dicarboxylic Acid	-1.2	
BC100208	CC1687-000	Cypermethrin; (R,S)-α-Cyano-3-phenoxybenzyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate; (1R)-(R)-cyano(3-phenoxy phenyl) methyl 3-(2,2-dichloro vinyl)-2,2- dimethylcyclopropane carboxylate	-5.9	

BC100209	CC1667-000	Deltamethrin; (S)-a-Cyano-3-phenoxybenzyl(1R)-cis-3-(2,2-dibromovinyl) -2,2-dimethyl cyclopropanecarboxylate	0.3		

2.0 Table of PTP Inhibitors tested in PTEN assay at 250uM. For select compounds, the IC50 value is recorded.

2.1 Diketone Phananthrolines

We are interested in exploring this series. **SF1720** (R=H) has low micromolar IC50. Attempts to derivatize the ring system are in progress.



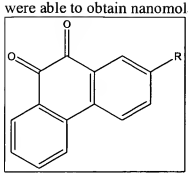
Notebook Number	Barcode Number	SF Number	Reference	Substitution (R)	IC50 (nM)
A053-38	BC100281	SF1720-000	A048-32-6	H	5000

We plan to optimize this series in a similar fashion to that of the Diketone Phenanthrenes. In addition, we feel that a non planer heterocyclic system (similar to benzyl), namely analogs of 1,2-di(pyridin-3-yl)ethane-1,2-dione, may have significant activity in the PTEN assay. We speculate that the pyridyl amines can coordinate with the protein via chelation to align the diketone functionality into the desired orientation producing an active series. Benzyl analogs, which lack coordination ability (**SF1729**) have little activity in the PTEN assay. We are pursuing the synthesis of 1,2-di(pyridin-3-yl)ethane-1,2-dione, and its analogs.

2.2 Diketone Phananthrenes

Another Diketone series that has activity in the PTEN assay is one where the diketone functionality is locked in a planer ring. Our first hit, **SF1670**, was quickly followed up by additional compounds designed to maximize interactions between the ligand and the protein. By adding aryl groups attached via a linker to the planer aromatic amine, we

were able to obtain nanomolar inhibitors of PTEN.



Notebook Number	Barcode Number	Sample Number	Reference	Substitution	IC50 (nM)
A033-90-3	BC100191	SF1670-000	A033-90-3	NHCOtBu	2000
A048-33-7	BC100257	SF1721-000	A048-33-7	H	3400
A048-33-8	BC100258	SF1722-000	A048-33-8	NO2	4000
A053-38	BC100281	SF1740-000	A053-28	NHCOCH2OPh	400/327*
A048-58-10	BC100293	SF1751-000	A048-57-1	NHCOCH2OPh(4-OMe)	622.6
A060-18	BC100315	SF1771-000	A048-78-1	NHCOCH2OPh(4-Me)	395.3
A060-22	BC100316	SF1772-000	A048-78-2	NHCOCH2OPh(2-iPr)	435.6
A060-60	BC100317	SF1773-000	A048-78-3	NHSO2Ph	221/297*
A060-74	BC100318	SF1774-000	A048-78-4	NHSO2Ph(4-NO2)	214.6
A048-48	BC100321	SF1777-000	A048-78-7	NHCOPh	291.7
A048-73	BC100323	SF1779-000	A048-78-9	NHCOPh(4-Me)	342.1
A060-78	BC100324	SF1780-000	A048-79-3	NHSO2Ph(4-tBu)	269
A060-92	BC100328	SF1784-000	A048-83-1	NHCOPh(2-NO2)	598.7
A060-96	BC100329	SF1785-000	A048-83-2	NHCO(CH2)3Ph	4998
A060-98	BC100330	SF1786-000	A048-83-3	NHCOCO2Et	692.4
A066-4B	BC100331	SF1787-000	A048-83-4	NHCOCH2OPh(2-OMe)	548.1
A066-6B	BC100332	SF1788-000	A048-83-5	NHCOCH2OPh(3-OMe)	410.4
A060-86	BC100333	SF1789-000	A048-83-6	NH2	776.1
A066-02	BC100334	SF1790-000	A048-83-7	NHCOCH2OPh(4-Cl)	2620

* Run multiple times.

As seen in the above table, our best hits have additional functionality linked to the diketone ring lowering the inhibition into the nanomolar range. We are continuing to optimize this series by examining the length and constituency of the linker unit as well as

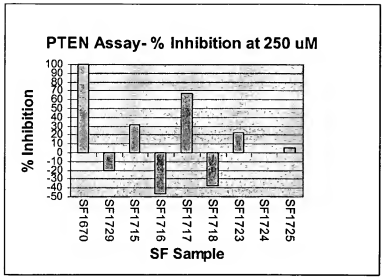
the choice of substituted aromatic ring on the end of the linker. In addition to aromatic rings, we are also planning aliphatic derivatives based on **SF1670**. We have examined the role of the diketone ring. It is our current belief that the diketone functionality needs to be locked in a planer orientation. As mentioned in the next section, a mono ketone in the phenanthrene ring system is also being explored **(CC1734)**.

2.3 Diketone Discussion

One interesting compound, **CC1670** (later renamed **SF1670**), had an initial IC50 in the single digit micromolar region. This compound was the first one in what we described as the Phenanthrene Diketone series. Researching this compound in the literature led us to several original papers describing how the compound was originally part of a library which exhibited CD45 inhibition. The researchers postulated that the diketone functionalities must be locked in a six member ring for suitable interactions with a CYS group in the active site. Several acyclic benzyl compounds, including benzyl **(SF1729)** were shown to exhibit little activity in the CD45 assay.

We decided to investigate compounds that resembled **SF1670** and **SF1729** and analogs in the PTEN assay. From the literature we were aware of the similarities of the active site in PTEN, CD45 as well as PTP1B. We were most interested in testing acyclic analogs of **SF1729** with the goal of obtaining selective inhibitors. We tested a series of acyclic analogs looking for activity in the PTEN assay, along with selectivity over CD45 and PTP1B.

In Graph 1, we have tabulated the results from the cyclic **(SF1670)** compound along with benzyl, the acyclic compound **SF1729,** and other acyclic analogs. Specifically the 4,4' di-Br, 3,3' di-MeO, 4,4' di-Me, 4,4' di-MeO, 4-OMe, 4-NH2, and 4-CONH2 analogs **(SF1715, SF1716, SF1717, SF1718, SF1723, SF1724, SF1725** respectively, Notebook page A048-32) were tested. The clear trend is that the acyclic compounds-as reported for the CD45 assay- did not show appreciable activity in the PTEN assay at 250 uM. In these compounds the two ketone functionalities are not restrained in a planer manner.



Graph 1: Initial Phenanthrene Diketone Series –Comparing Cyclic and Acyclic Analogs in the PTEN Assay at 250 uM

Researching structures similar to **SF 1670** we discovered a different series, which we labeled the Phenanthroline Diketone series. One of these, **SF1720** (Notebook page A048-32-6) showed similar activity as **SF1670** in the PTEN assay. The two structures are sterically similar however, are stereoelectronically very different. An analogy to visualize these distinct differences, can be to examine a simple benzene and pyridine ring-similar in shape but with profoundly different stereoelectronics. The Phenanthroline Diketone series has proven more difficult to derivatize, however, attempts are still in progress for further derivatives due to the preliminary selectivity results.

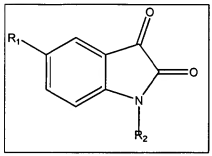
We have started optimizing the Phenanthrene Diketone series discovered in our labs. Starting from **SF1670** with an IC50 value of 2uM we have optimized this activity into the nanomolar range. We have manipulated the initial compound by inserting additional aromatic ring(s) attached via a linker. We have modified both the length and the structure of the linkers. We are currently attempting to optimize for selectivity with other phosphatases based on the crystal structure of the active site of PTEN protein.

3.0 Miscellaneous Dicarbonyl Compounds

3.1 Isatin Compounds

Based on the observed activity of the diketones, we investigated the Isatin Series (alpha

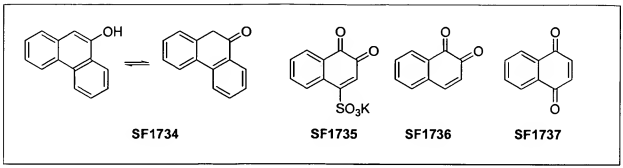
keto amides). These compounds have modest micromolar activity in the PTEN assay. We have just started SAR work on the series and plan to add functionality in three specific areas on the template molecule. Namely, attaching exploitable functionality on the aromatic ring, derivtizing via the amide NH group, and reacting the cyclic ketone to yield imine or olefinc derivatives. We have found that manipulation of 5-nitroindoline-2,3-dione (**SF1770**) by reducing the nitro group on the aromatic ring and attaching aryl groups via amide linkers, and by adding additional functionality on the amide nitrogen, can effect the inhibition of these compounds in the PTEN assay. The initial compounds synthesized and purchased exhibit low micromolar activity. The IC50 for our two best compounds to date (**SF1770** and **SF1773**) are in the double digit micromolar range.



notebook_number	barcode_Number	SF Number	R1	R2	IC50 (uM)
A048-66-20	BC100313	SF1770-000	5-NO2	H	2.2
A048-70	BC100322	SF1778-000	5-(NHCOPh(4-Me))	H	22.05
A048-77	BC100325	SF1781-000	5-(NHCOCH2OPh)	H	18.9
A048-79-2	BC100327	SF1783-000	5-NO2	CH2Ph(2,4-diCl)	4.6
A048-83-8	BC100335	SF1791-000	5-H	CH2Ph(4-Me)	>250
A048-83-9	BC100336	SF1792-000	5-H	SO2Ph(4-F)	>250
A048-83-10	BC100337	SF1793-000	5-Me	CH2Ph(4-Cl)	>250
A048-83-11	BC100338	SF1794-000	5-iPr	H	>250
A048-83-12	BC100339	SF1795-000	5-Br	Et	100.459

3.2 Miscellaneous Diketone Compounds

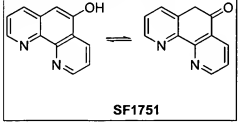
To determine what role the diketones played in PTEN activity, we have begun to examine a variety of dicarbonyl compounds and have discovered significant PTEN activity at 250uM. These compounds all exhibited low double digit micromolar IC50 activity in the PTEN assay.



SF1734 SF1735 SF1736 SF1737

Compound	PTEN Ic50
CC1734	33uM
CC1735	20uM
CC1736	21uM
CC1737	43uM

We plan to derivatize the **SF1734** series in a similar fashion to that described for the Diketone series. In addition, we plan to functionalize the center ring system. One interesting observation has been the comparison of PTEN activity of **SF1734** and **SF1751**. It appears the heterocyclic compound as little activity at 250uM while the all carbon ring system (**SF1734**) has an IC50 value of 33uM. It could be that the stereoelectronics of the heterocyclic ring system is pushing the equilibrium to the enol form and thus reducing activity in the PTEN assay. We plan to make analogs of **SF1734** which contain both electron withdrawing and electron donating substitution on the core ring to test this hypothesis.



SF1751

Because PTEN acts against PI3 kinase we believe that PTEN inhibitors will allow for efficient PI3 kinase activity in cells which will impart beneficial characteristics as hypothesized herein.

4.0 PTEN Use/ Utility

- PTEN inhibitors may protect against septic shock. The following article supports this idea. The authors summarize that there work suggests that stimulation of the PI3K pathway may be an effective approach for preventing or treating sepsis and/or septic shock.

Williams, David L.; Li, Chuanfu; Ha, Tuanzhu; Ozment-Skelton, Tammy; Kalbfleisch, John H.; Preiszner, Johanna; Brooks, Lynne; Breuel, Kevin; Schweitzer, John B. Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis *Journal of Immunology* **2004,** 172(1), 449-456

ABSTRACT

We examined the effect of modulating phosphoinositide 3-kinase (PI3K) activity in a murine model of cecal ligation and puncture-induced polymicrobial sepsis. Inhibition of PI3K activity with wortmannin increased serum cytokine levels and decreased survival time in septic mice. We have reported that an immunomodulator, glucan phosphate, induces protection in murine polymicrobial sepsis. We observed that glucan stimulated tissue PI3K activity, which positively correlated with increased survival in septic mice. We investigated the effect of PI3K inhibition on survival in septic mice treated with glucan. Treatment of mice with the PI3K inhibitors, wortmannin and >LY294002<, completely eliminated the protective effect of glucan, indicating that protection against septic mortality was mediated through PI3K. Inhibition of PI3K resulted in increased serum levels of IL1-beta, IL-2, IL-6, IL-10, IL-12, and TNF-alpha in septic mice. Apoptosis is thought to play a central role in the response to septic injury. We observed that inhibition of PI3K activity in septic mice resulted in increased splenocyte apoptosis and a change in the anatomic distribution of splenocyte apoptosis. We conclude that PI3K is a compensatory mechanism that suppresses proinflammatory and apoptotic processes in response to sepsis and/or inflammatory injury. Thus, PI3K may play a pivotal role in the maintenance of homeostasis and the integrity of the immune response during sepsis. We also observed that glucan phosphate decreased septic morbidity and mortality through a PI3K-dependent mechanism. This suggests that stimulation of the PI3K pathway may be an effective approach for preventing or treating sepsis and/or septic shock.

- PTEN inhibitors may be useful in therapeutic angiogenesis. Sustained release of PTEN inhibitors (via nanoparticle technology or other slow in vivo release) may lead to therapeutic angiogenesis. See following article.

Yeh, J. L..,Giordano, F. J., Gene-based therapeutic angiogenesis. *Semin Thorac Cardiovasc Surg* **2003**, *15*,. 236-249

ABSTRACT

Stimulating new blood vessel growth in ischemic hearts or limbs is a

hopeful new approach for patients with advanced vascular disease. This approach is based generally upon the hypothesis that sufficient exposure of a vascular bed to an >angiogenic< protein will stimulate neovascularization. Most >angiogenic< proteins have a markedly short serum half-life. To overcome this, researchers **have turned to gene therapy to ensure continuous expression of >angiogenic< proteins and prolonged exposure in the targeted vascular beds**. This field is still evolving, and although early >clinical< >trial< results suggest >angiogenic< gene therapy can be successful, many questions remain. As we continue to learn more about the complex interplay and coordinated action of the various factors involved in regulating >angiogenesis<, it is likely that strategies for therapeutic >angiogenesis< will continue to change. This review addresses the current state of >angiogenic< gene therapy, contrasts gene therapy with >angiogenic< protein delivery, describes early and recent >clinical< >trial< data, and discusses potential new directions in the field.;

More therapeutic angiogenesis (ie possible use for PTEN inhibitors):

Kleiman, Neal S.; Patel, Nirav C.; Allen, Keith B.; Simons, Michael; Yla-Herttuala, Seppo; Griffin, Elaine; Dzau, Victor J. Evolving revascularization approaches for myocardial ischemia. *American Journal of* Cardiology 2003, 92(9B), 9N-17N

ABSTRACT

Stable angina pectoris secondary to ischemic heart disease is a common and disabling condition. Medical therapy aims to relieve symptoms, improve exercise capacity, and decrease cardiac events by reducing myocardial oxygen demand or improving coronary blood supply to the ischemic myocardium. If medical treatment is inadequate, invasive revascularization procedures to improve coronary perfusion are considered. Percutaneous transluminal coronary angioplasty (PTCA) and coronary artery bypass graft (CABG) surgery are well-established and widely used myocardial revascularization techniques. Recent advances in PTCA have attempted to address the problem of restenosis, initially through the deployment of bare metal intracoronary stents and, more recently, with drug-eluting stents. Developments in CABG have focused on reducing the invasiveness of the procedure and minimizing the incidence of serious complications. Refinements include the use of mechanical stabilizers, endoscopic harvesting of conduit vessels, robotic telemanipulation systems, and fully automated anastomotic devices. Surgical laser transmyocardial revascularization and therapeutic >angiogenesis< represent newer approaches to coronary revascularization. Therapeutic >angiogenesis< aims to deliver an >angiogenic< growth factor or cytokine to the myocardium to stimulate collateral blood vessel growth throughout the ischemic tissue. The >angiogenic< factor may be administered as a recombinant protein or as a transgene within a plasmid or gene-transfer vector. Ongoing >angiogenic< gene therapy >clinical< >trials< are evaluating which factors, vectors, and delivery techniques hold the greatest promise for management of patients with chronic stable angina.

- Antisense PTEN inhibitor for diabetic use.

http://www.genetrove.com/inVivoGT.html

These data demonstrate that PTEN antisense will sensitize tissues to insulin resulting in decreased blood glucose concentrations in diabetic mice, but will not affect blood glucose levels in normal mice.

- Protection of cells is not guaranteed if p53 is inhibited.

Bonini, P.; Cicconi, S.; Cardinale, A.; Vitale, C.; Serafino, A. L.; Ciotti, M. T.; Marlier, L. N. Oxidative stress induces p53-mediated apoptosis in glia: p53 transcription-independent way to die. *J Neurosc.i Res.* **2004** (75), 83-95

ABSTRACT

Oxidative stress has been implicated in the pathogenesis of stroke, traumatic brain injuries, and neurodegenerative diseases affecting both neuronal and glial cells in the central nervous system (CNS). The tumor suppressor protein p53 plays a pivotal function in neuronal apoptosis triggered by oxidative stress. We investigated the role of p53 and related molecular mechanisms that support oxidative stress-induced apoptosis in glia. For this purpose, we exposed C6 glioma cells and primary cultures of rat cortical astrocytes to an H(2)O(2)-induced oxidative stress protocol followed by a recovery period. We evaluated the effects of >pifithrin-alpha< (PF-alpha), which has been reported to protect neurons from ischemic insult by specifically inhibiting p53 DNA-binding activity. Strikingly, PF-alpha was unable to prevent oxidative stress-induced astrocyte apoptosis. We demonstrate that p53 is able to mediate an apoptotic response by direct signaling at mitochondria, despite its transcriptional activity. The z-VAD-fmk-sensitive apoptotic response requires a caspase-dependent MDM-2 degradation, leading to p53 mitochondrial targeting accompanied by cytochrome c release and nucleosomal fragmentation.;

- Combination steroid treatment or inhibition in combo with PTEN inhibitors. (Endometreosis market for PTEN agonisits?) See following article.

Guzeloglu-Kayisli, Ozlem; Kayisli, Umit A.; Al-Rejjal, Rafat; Zheng, Wenxin; Luleci, Guven; Arici, Aydin Regulation of PTEN (phosphatase and tensin homolog deleted on chromosome10) expression by estradiol and progesterone in human endometrium . *Journal of Clinical Endocrinology & Metabolism* **2003**, *88(10)*, 5017-5026

ABSTRACT

PTEN (phosphatase and tensin homolog deleted on chromosome 10) is a

>tumor< suppressor gene, mutated frequently in a variety of human >tumors<. PTEN regulates cell growth, apoptosis, and proliferation. Phosphorylation in PTEN tail causes its inactivation and decreases its degradation. There is little known about the regulation of PTEN by ovarian steroids. We hypothesized that PTEN expression in human endometrium is variable throughout the menstrual cycle and early pregnancy, and that ovarian steroids regulate PTEN expression because PTEN is critical in many steroid-sensitive tissues such as endometrium, prostate, and breast. In the present study, we have observed a direct regulation of PTEN by ovarian steroids. Estradiol increased PTEN phosphorylation at 5-15 min. After 24-h treatment, progesterone induced a significant increase in PTEN protein levels, assessed by Western blot. Furthermore, we evaluated for the first time a comparison between menstrual cycle and early pregnancy, immunohistochemically. Endometrial PTEN expression revealed temporal and spatial changes throughout the menstrual cycle and during early pregnancy. We conclude that estradiol may downregulate PTEN activity by increasing its phosphorylation, but progesterone is likely to regulate the PTEN pool by decreasing its phosphorylation and increasing its protein level. Presented data, therefore, suggest that ovarian steroids regulate the endometrial PTEN pool. We propose that PTEN might be one of the signaling proteins that estrogen and progesterone are acting to affect endometrial cell proliferation and/or apoptosis.

- Possible use as notch ligand as targeting agent for PTEN inhibitors

Jundt, Franziska; Proebsting, Kristina Schulze; Anagnostopoulos, Ioannis; Mathas, Stephan; Stein, Harald; Doerken, Bernd Activated Notch signaling might be a novel therapeutic target for multiple myeloma. *Blood* **2003**, 11, 928a

ABSTRACT

Notch signaling plays a key role in the development and differentiation of various hematopoietic lineages. In the hematopoietic system, Notch receptors are expressed in early hematopoietic stem cells, whereas Notch ligands are found in bone marrow stroma, which provides the microenvironment necessary for stem cell survival and differentiation. In addition, we recently demonstrated that Notch signaling is involved in the pathogenesis of B-cell-derived tumor cells of Hodgkin lymphoma (Blood. 2002;99:3398-3403). We described a novel mechanism for the oncogenic capacity of Notch by showing that interactions of overexpressed intact Notch1 and Notch2 receptors on tumor cells with their cognate ligand Jagged1 dramatically induce both proliferation and inhibition of apoptosis in vitro. We further provided evidence that in Hodgkin lymphoma Jagged1 is expressed in malignant as well as in bystander cells co-localizing with Notch-positive tumor cells. Notch signaling may therefore be activated in tumor cells by Jagged1 through homotypic or heterotypic cell-cell interactions and it seems likely that these interactions also contribute to lymphomagenesis in vivo. However, a pathogenetic role for Notch in multiple myeloma (MM), where tight interactions between neoplastic plasma cells and their microenvironment are essential for tumor cell growth, is currently unknown. In this study, we therefore investigated Notch gene expression in cultured and primary multiple myeloma cells. To that end, we analyzed 14 cases of MM for expression of Notch1 and Notch2 by immunohistochemistry. In all cases Notch1 and Notch2 were highly expressed

in MM cells. Strong Notch expression in MM cells was comparable to tumor cells of classic Hodgkin lymphoma, that we analyzed in our recent study. In contrast, we found low to undetectable levels of Notch1 and Notch2 in plasma cells of bone marrow of normal donors and in plasma cells of reactive lymphoid tissue. To verify high expression of Notch1 and Notch2 in cultured MM cells, we performed Western blot analysis of five MM cell lines. According to our data in primary MM cells, we found that both Notch receptors were highly expressed in all MM cell lines. However, freshly isolated CD19+ B cells and CD19+ B cells, that we differentiated to CD38+ plasmablastic cells in vitro, were almost completely devoid of Notch expression. Our data indicate that cultured and primary MM cells differ from their non-neoplastic counterparts with respect to strong Notch1 and Notch2 expression. Our data further provide evidence that **ligand-induced Notch signaling is a novel growth factor for multiple myeloma cells and suggest that these interactions contribute to lymphomagenesis of multiple myeloma in vivo**. Studies are under way to block Notch signaling by gamma-secretase inhibitors to further determine its role in tumor cell proliferation and resistance towards apoptosis in MM.

- PTEN inhibitors could help neural stem cell self-renewal

Erickson, R. I.; Groszer, M.; Ngo, C.; Liu, X.; Wu, H.; Kornblum, H.I. Serial passages of cortical neurospheres from conditional Pten mutant mice demonstrate persistent effects on neural stem cell self - renewal.- Society for Neuroscience Abstract Viewer and Itinerary Planner VOL. 2003 2003 PP. Abstract No. 243.18

ABSTRACT

Pten is a lipid phosphatase that acts as a >tumor< suppressor gene and is frequently mutated in gliomas. As one of its primary actions, Pten inhibits the PI3K/Akt pathway; therefore cells that have lost Pten function are enlarged, have enhanced survival, and increased proliferative capacity. Conditional knockout of Pten in nestin-containing cells results in enlarged, disorganized brains, and increased BrdU labeling in ventricular zones (Groszer et al. 2001). Using the neurosphere (NS) culturing system, we showed that Pten mutant (MUT) cells had increased proliferation mediated by more rapid cycling, as well decreased apoptosis. Neural stem cells (NSC) were more capable of self-renewal for at least one passage, but it **is not known whether this effect persists through multiple passages.** In the current study, we examine the effects of Pten deletion on multiply passaged neurospheres to determine whether effects on proliferation and self-renewal persist over multiple passages and whether there is an effect on cell fate specification. Cells from E14 MUT and wildtype (WT) cortices were plated at 5000 cells per ml and passaged several times. At each passage, aliquots of NS from each individual embryo were counted, measured, differentiated, and immunostained for neurons, astrocytes and oligodendrocytes. The number of NS generated remained higher in MUT over many passages, while mutant diameter measurements merged closer to WT. **The percent of tri-potential NS as well as the ratio of neurons/total cell counts remained higher in MUT**. This suggests that, **although proliferation slows in MUT, there are more NSC going through self-renewing divisions over subsequent passages**

More on PTEN and stem cells

Groszer, M.; Erickson, R.; Scripture-Adams, D. D.; Lesche, R.; Trumpp, A.; Zack, J. A.; Kornblum, H. I.; Liu, X.; Wu, H. Negative regulation of neural stem/progenitor cell proliferation by the Pten tumor suppressor gene in vivo. *Science*, VOL. 294 NO. 5549 **2001** Dec 7 PP. 2186-9

ABSTRACT

The mechanisms controlling neural stem cell proliferation are poorly understood. Here we demonstrate that the PTEN >tumor< suppressor plays an important role in regulating neural stem/progenitor cells in vivo and in vitro. Mice lacking PTEN exhibited enlarged, histoarchitecturally abnormal brains, which resulted from increased cell proliferation, decreased cell death, and enlarged cell size. Neurosphere cultures revealed a greater proliferation capacity for tripotent Pten-/- central nervous system stem/progenitor cells, which can be attributed, at least in part, to a shortened cell cycle. However, cell fate commitments of the progenitors were largely undisturbed. Our results suggest that PTEN negatively regulates neural stem cell proliferation.

- PTEN inhibitors for preventing neurodegenerative disease

Waldmeier, Peter C.; Tatton, William C., Interrupting apoptosis in neurodegenerative disease: Potential for effective therapy? *Drug Discovery Today* VOL. 9 NO. 5 1 March, 2004 PP.210-218

ABSTRACT

Current treatment options for neurodegenerative diseases are limited and mainly affect only the symptoms of disease. Because of the unknown and probably multiple causes of these diseases, they cannot be readily targeted. However, it has been established that apoptosis contributes to neuronal loss in most neurodegenerative diseases. A possible treatment option is to interrupt the signaling networks that link neuronal damage to apoptotic degradation in neurodegeneration. The viability of this option depends upon the extent to which apoptosis accounts for neuron loss, whether or not interruption of apoptosis signaling results in recovery of neurological function and whether or not there are significant downsides to targeting apoptosis. Several compounds acting at different sites in known apoptotic signaling networks are currently in development and a few are in clinical trial. **If an apoptosis-targeted compound succeeds in slowing or halting neurological dysfunction in one or more neurodegenerative diseases, a new era in the treatment of neurodegenerative diseases will begin.**

- Possible use for the prevention of apoptosis in mature erythroid progenitor cells.

Here is some important info; by inference a PTEN inhibitor would act opposite the effects noted here for the LY compound on erythroid progenitor

Paiboonsukwong, K.; Choi, I.; Matsushima, T.; Abe, Y.; Nishimura, J.; Winichagoon, P.; Fucharoen, S.; Nawata, H.; Muta, K. The signaling pathways of erythropoietin and

interferon-gamma differ in preventing the apoptosis of mature erythroid progenitor cells. Int J Hematol VOL. 78 2003 Dec PP. 421-8

Interferon (IFN)-gamma is a survival factor for mature erythroid progenitor cells. To elucidate related survival mechanisms, we compared the role of phosphatidylinositol 3-kinase (PI3-kinase) in the survival signals of IFN-gamma and erythropoietin (EPO). Human erythroid colony-forming cells (ECFCs) purified from peripheral blood were used, and >Ly294002< was used as a PI3-kinase inhibitor. Treating ECFCs with a high concentration of >Ly294002< (50 micromol/L) in the presence of EPO and/or IFN-gamma reduced cell viability by inducing apoptosis. However, treating cellswith a lower concentration of >Ly294002< (10 micromol/L) did not affect the antiapoptotic function of IFN-gamma and abolished the antiapoptotic effect of EPO. Adding IFN-gamma or EPO induced Bcl-x expression in ECFCs, as determined by Western blotting, and expression was suppressed in the presence of >Ly294002<. We also examined the phosphorylation of the protein kinase Akt, the downstream target of PI3-kinase. EPO stimulation significantly increased the level of Akt phosphorylation, but IFN-gamma did not. These results suggest that IFN-gamma plays a role in preventing the apoptosis of erythroid progenitor cells by affecting Bcl-x expression, thereby reducing the disruption of the mitochondrial transmembrane potential via PI3-kinase pathways that are related to but distinct from the EPO pathway

---

- Possible anti obesity therapeutic area. We expect our PTEN inhibitors would increase the effect of leptin in vivo by activating the PI3 kinase pathway

Huang, Wan; Dedousis, Nikolas; Bhatt, Bankim A.; O'Doherty, Robert M., Impaired activation of phosphatidylinositol 3-kinase by leptin is a novel mechanism of hepatic leptin resistance in diet-induced obesity - Journal of Biological Chemistry VOL. 279 NO. 21 May 21, 2004 PP. 21695-21700

ABSTRACT

Obesity is associated with the development of leptin resistance. However, the effects of leptin resistance on leptin-regulated metabolic processes and the biochemical defects that cause leptin resistance are poorly understood. We have addressed in rats the effect of diet-induced obesity (DIO), a situation of elevated tissue lipid levels, on the well described lipid-lowering effect of leptin in liver, an action that is proposed to be important for the prevention of tissue lipotoxicity and insulin resistance. In addition, we have addressed the role of phosphatidylinositol 3-kinase (PI 3-kinase) in mediating the acute effects of leptin on hepatic lipid levels in lean and DIO animals. A 90-min leptin (dollar sign 10 ng/ ml) perfusion of isolated livers from lean animals decreased triglyceride levels by 42 +/- 5% (p = 0.006). However, leptin concentrations ranging from dollar sign 10 to dollar sign 90 ng/ml had no effect on triglyceride levels in livers from DIO animals. The acute lipid-lowering effect of leptin on livers from lean animals was mediated by a PI 3- kinase-dependent mechanism, **because wortmannin and >LY294002<, the PI 3- kinase inhibitors, blocked the effects of leptin on hepatic**

triglyceride levels and leptin increased liver PI 3-kinase activity by 183 +/- 6% (p = 0.003) and insulin receptor substrate 1 tyrosine phosphorylation by 185 +/- 30% (p = 0.02) in the absence of PI 3-kinase inhibitors. Contrary to the effects of leptin in lean livers, leptin did not activate PI 3-kinase in livers from DIO rats. These data present evidence for a role for 1) leptin resistance in contributing to the excessive accumulation of tissue lipid in obesity, 2) PI 3-kinase in mediating the acute lipid-lowering effects of leptin in liver, and 3) defective leptin activation of PI 3-kinase as a novel mechanism of leptin resistance.

- PTEN inhibitors may block detrimental caspase-3 damage. Useful in diabetes and kidney failure.

Gao, Yong Mei; Debigare, Richard; Meireles, Christiane; Bailey, James L; Price, S. Russ Insulin suppresses caspase-3-mediated actin cleavage and muscle proteolysis in L6 muscle cells: Implications for muscle atrophy-FASEB Journal VOL. 18 NO. 4-5 2004 PP. Abst. 827.8.

Muscle atrophy in catabolic conditions (e.g., diabetes, kidney failure) results from accelerated protein degradation (PD) by the ubiquitin-proteasome (Ub-P) system. Insulin resistance may be a signal for increased PD because: 1) insulin suppresses PD in muscle; and 2) insulin insufficiency stimulates muscle PD. We found that caspase-3 cleaves actin into fragments that are degraded by the Ub-P system in muscle. To examine the relationships between insulin, proteolysis, caspase-3 and actin cleavage in muscle, L6 muscle cells were incubated in medium with 0.5% serum +/- insulin as a model of insulin insufficiency. Serum deprivation (SD) increased PD 17% (P<0.05), induced caspase-3 activity and increased the cleavage actin. Insulin attenuated these responses but the inhibitory effects required treatment for >4 h. In SD cells, insulin did not reduce the amount of procaspase-3 protein but increased several inhibitors of apoptosis (IAP) proteins which interact with caspase-3. Addition of >LY294002<, an inhibitor of PI 3-kinase, partially blocked the insulin-induced suppression of PD, and actin cleavage. In muscle of rats with kidney failure or acute diabetes, caspase-3 activity was higher and actin fragments were more abundant than in pair-fed, control rats. These data indicate that **insulin acts through the PI 3-kinase and other pathways to regulate caspase-3 activity and PD in muscle.** Our findings also suggest that insulin resistance is a stimulus for proteolysis in muscle.

- Inhibition of PTEN thus should enhance PDGF response and promote angiogenesis and other PDGF related activities.

Mahimainathan, L.; Choudhury, G. G. Inactivation of platelet-derived growth factor receptor by the tumor suppressor PTEN provides a novel mechanism of action of the phosphatase. -J Biol Chem VOL. 279 NO. 15 2004 Apr 9 PP. 15258-68

ABSTRACT

PTEN, mutated in a variety of human >cancers<, is a dual specificity protein phosphatase and also possesses D3-phosphoinositide phosphatase activity on phosphatidylinositol 3,4,5-tris-phosphate (PIP(3)), a product of phosphatidylinositol 3-kinase. This PIP(3) phosphatase activity of PTEN contributes to its >tumor< suppressor function by inhibition of Akt kinase, a direct target of PIP(3). We have recently shown that Akt regulates PDGF-induced DNA synthesis in mesangial cells. In this study, we demonstrate that expression of PTEN in mesangial cells inhibits PDGF-induced Akt activation leading to reduction in PDGF-induced DNA synthesis. As a potential mechanism, we show that PTEN inhibits PDGF-induced protein tyrosine phosphorylation with concomitant dephosphorylation and inactivation of tyrosine phosphorylated and activated PDGF receptor. Recombinant as well as immunopurified PTEN dephosphorylates autophosphorylated PDGF receptor in vitro. Expression of phosphatase deficient mutant of PTEN does not dephosphorylate PDGF-induced tyrosine phosphorylated PDGF receptor. **Rather its expression increases tyrosine phosphorylation of PDGF receptor. Furthermore, expression of PTEN attenuated PDGF-induced signal transduction including phosphatidylinositol 3-kinase and Erk1/2 MAPK activities. Our data provide the first evidence that PTEN is physically associated with platelet-derived growth factor (PDGF) receptor and that PDGF causes its dissociation from the receptor. Finally, we show that both the C2 and tail domains of PTEN contribute to binding to the PDGF receptor. These data demonstrate a novel aspect of PTEN function where it acts as an effector for the PDGF receptor function and negatively regulates PDGF receptor activation.**

- Inhibition of PTEN will promote PI3K activity and protect from reperfusion injury.

Boucher, M.; Pesant, S.; Falcao, S.; de Montigny, C.; Schampaert, E.; Cardinal, R.; Rousseau, G. Post-ischemic cardioprotection by A2A adenosine receptors: dependent of phosphatidylinositol 3-kinase pathway *J. Cardiovasc Pharmacol* 43(3) **2004** Mar, 416-22

ABSTRACT

Activation of myocardial A2A adenosine receptors during reperfusion has been shown to be cardioprotective. The intracellular mechanisms underlying this protection remain unknown. To understand the beneficial effects of activated A2A adenosine receptors in such a state, we investigated whether the enzymes phosphatidylinositol 3-kinase (PI3K) and caspase-3 can account for this post-ischemic cardioprotective effect in an anesthetized rabbit model of myocardial infarction (30 minutes ischemia; 5 hours reperfusion). Administration of the A2A agonist CGS21680 (0.2 microg/kg/min) 5 minutes before reperfusion began (Early) reduced infarct size expressed as a percentage of the area at risk (25.7 +/- 5.3% versus 46.5 +/- 5.3% for the control group; * P 60 0.05). Treatment with the A2A agonist 5 minutes after the onset of reperfusion (Late) had no effect on infarct size (38.2 +/- 6.2%). In the presence of a selective inhibitor of PI3K (>LY294002<), the beneficial effects of CGS21680 on infarct size was no longer observed (43.9 +/- 7.9%). After 5 hours of reperfusion, higher PI3K activity in the ischemic region was observed in the Early group compared with the other experimental groups. Caspase-3 activity was not observed in these

different groups. In another set of experiments, PI3K activity was significantly higher during the first 15 minutes of reperfusion in the Early group as compared with the Control group. Caspase-3 activity increased rapidly during the first 15 minutes of reperfusion in the Control group and remained stable in the Early group. These results indicated that post-ischemic cardioprotection afforded by A2A adenosine receptor activation is PI3K-dependent and modulate rapidly other signaling pathways such as caspase-3.

- PTEN and stem cells.

Our PTEN inhibitors may be able to allow stem cells to proliferate without differentiation and then use in combo with 2-(anilino)-4-aminopyrimidines types of molecules to then start differentiation

(Wu, X.; Ding, S.; Cing, Q.; Gray, N.S.; Schultz, P. G. Journal American Chemical Society 2004, 126, 1590-1591)

Stem cells are multipotent cells with the ability to self-renew and differentiate into specialized cells in response to appropriate signals.1 Most tissues have endogenous stem/progenitor cells which, upon injury to the organ, can proliferate and differentiate at the damaged site. The adult heart is composed mainly of postmitotic and terminally differentiated cells. Although a subpopulation of myocardial cells with cardiac stem cell character was identified recently, their limited availability hinders therapeutic applications.2 Stem cells derived from other tissues, such as bone marrow, have been shown to be capable of repairing heart damage in animal models,3 but inefficient differentiation and possible fusion with somatic cells limit their use in cardiac repair.4 Pluripotent embryonic stem (ES) cells represent a possible unlimited source of functional cardiomyocytes. However, the in vitro differentiation of ES cells into cardiomyocytes involves a poorly defined, inefficient, and relatively nonselective process.5 Consequently, the development of new approaches for the directed differentiation of ES cells into cardiomyocytes will likely facilitate therapeutic application of ES cells in heart disease, as well as provide important tools for probing the molecular mechanism of cardiomyocyte differentiation and heart development.

- Possible method to prevent hypoxia driven damage during and after surgery. PTEN inhibitors may enhance preconditioning in humans prior to surgery.

Carini, R; De Cesaris, MG; Splendore, R; Baldanzi, G; Nitti, MP; Alchera, E; Filigheddu, N; Domenicotti, C; Pronzato, MA; Graziani, A; Albano, E.; Role of phosphatidylinositol 3-kinase in the development of hepatocyte preconditioning. GASTROENTEROLOGY, 127 (3): 914-923 SEP 2004

Abstract:

Background & Aims: Ischemic preconditioning has been proved effective in reducing ischemia/reperfusion injury during liver surgery. However, the mechanisms involved are still poorly understood. Here, we have

investigated the role of phosphatidylinositol 3-kinase (PI3K) in the signal pathway leading to hepatic preconditioning. Methods: PI3K activation was evaluated in isolated rat hepatocytes preconditioned by 10-minute hypoxia followed by 10-minute reoxygenation. Results: Hypoxic preconditioning stimulated phosphatidylinositol-3,4,5-triphosphate production and the phosphorylation of PKB/Akt, a downstream target of PI3K. Conversely, PI3K inhibition by wortmannin or *LY294002* abolished hepatocyte tolerance against hypoxic damage induced by preconditioning. PI3K activation in preconditioned hepatocytes required the stimulation of adenosine A(2A) receptors and was mimicked by adenosine A(2A) receptors agonist CGS21680. In the cells treated with CGS21680, PI3K activation was pre! vented either by inhibiting adenylate cyclase and PKA with, respectively, 2,5-dideoxyadenosine and H89 or by blocking Galphai-protein and Src tyrosine kinase with, respectively, pertussis toxin and PP2. H89 also abolished the phosphorylation of adenosine A(2A) receptors. However, the direct PKA activation by forskolin failed to stimulate PI3K. This suggested that PKA-phosphorylated adenosine A(2A) receptors may activate PI3K by coupling it with Galphai-protein through Src. We also observed that, by impairing PI3K-mediated activation of phospholypase Cgamma (PLCgamma), wortmannin and *LY294002* blocked the downstream transduction of preconditioning signals via protein kinase C
(PKC) delta/e isozymes. Conclusions: PI3K is activated following hepatocyte hypoxic preconditioning by the combined stimulation of adenosine A(2A) receptors, PKA, Galphai protein, and Src. By regulating PKC-epsilon/delta-dependent signals, PI3K can play a key role in the development of hepatic tolerance to hypoxia/reperfusion.

CLAIMS

1. A method of increasing neovascularization comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

2. A method of preventing cell injury comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

3. A method of preventing cell death comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

4. A method of converting cancer cells into a more treatable state comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

5. A method of converting cancer stem cells to a state whereby they can then be effectively treated with other drugs comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

6. A method of treating diabetes comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

7. A method of expanding stem cells without inducing lineage commitment comprising incubating stem cells with an effective amount of a composition comprising a PTEN inhibitor described herein.

8. A method of protecting against septic shock comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

9. A method of therapeutic angiogenesis comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

10. A method of neural stem cell self renewal comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

11. A method of preventing neurodegenerative disease comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

12. A method of preventing apoptosis comprising administering to a patient in

need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

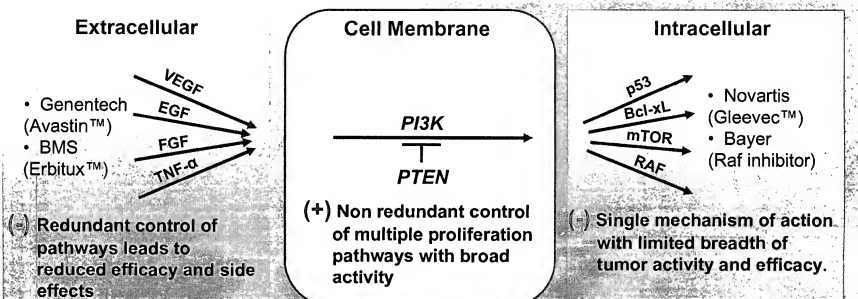
13. A method of treating obesity comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

14. A method of blocking detrimental caspase-3 damage comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

15. A method of preventing hypoxia driven damage comprising administering to a patient in need thereof an effective amount of a composition comprising a PTEN inhibitor described herein.

PI3K/PTEN Emerging Pathway Biology

Targeting the core ***PI3K/PTEN*** pathway has significant therapeutic advantages over upstream extracellular and downstream intracellular targets

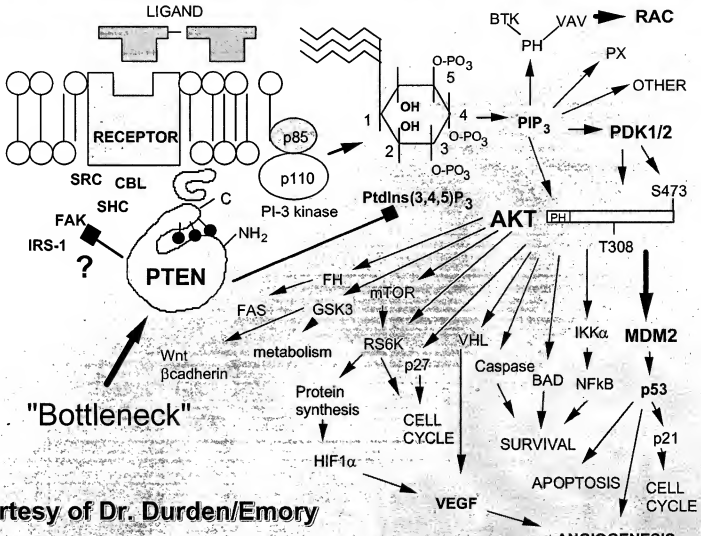


■Semafore Therapeutic Platforms

- ■*PI3K Inhibition*
- ■*PTEN Inhibition*

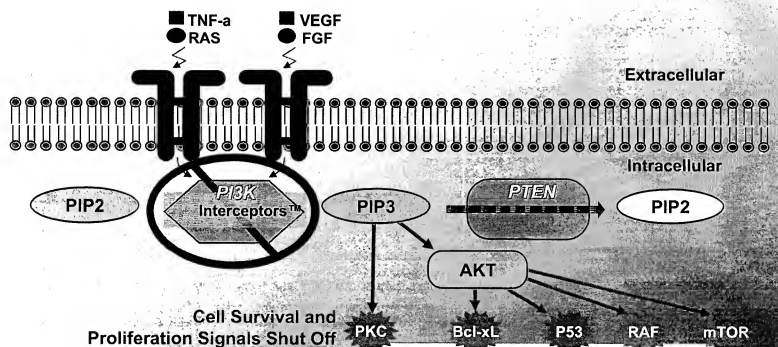
BEST AVAILABLE COPY

PTEN/PI3K Signaling Axis Detail



PI3K Inhibition Platform - Interceptors

Semafore's first core therapeutic platform is based on inhibiting the ***PI3K/PTEN*** pathway by inhibiting ***PI3K*** leading to regulated death of diseased cells



Therapeutic Applications

- Cancer
- Macular degeneration
- Inflammatory diseases
- Coronary artery disease

Summary of PI3 Kinase Inhibitor In Vivo Studies-SF1126

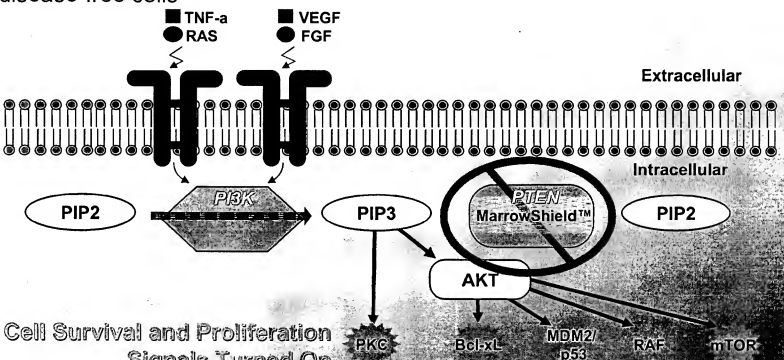
SF1126: Targeted Prodrug PI3K Inhibitor
Demonstrated Xenograft Efficacy
Well tolerated drug (i.v; s.c; i.m.)

Glioma	U87MG- PTEN null ; p53 wild type	[90% Inhib]
NSCL	H1299 – PTEN wild type; p53 null	[68%]
Prostate	PC-3 –PTEN null; p53 null	[74%]

SF1126: Preclinical studies in progress
First in man Phase I clinical-- 2005

PTEN Inhibition Platform

Semafore's second core therapeutic platform is based on promoting the ***PI3K/PTEN*** pathway by inhibiting ***PTEN*** leading to preservation of healthy disease-free cells



PTEN INTRODUCTION

PTEN = Phosphatase and tensin homologue deleted on chrmosome 10 (MMAC)

55-kDa (403 aa) dual specificity protein (poor) and lipid phosphatase(good)
NH2-terminal catalytic domain;
COOH-terminal C2 domain
(with lipid-binding and membrane targeting functions)

Acts as a tumor suppressor by dephosphylation of PI(3,4,5)P3 [D3 position]
(PTEN is antagonist of PI3K)

PTEN helps regulate:
- cell survival
- proliferation
- growth
- motility
- angiogenesis

PTEN regulated by:
- transcription
- PO4 dependent stability
- PDZ domain interactions
- redox conditions (reversible)
- (disulfide C124—C71)

POTENTIAL APPLICATIONS OF PTEN INHIBITORS

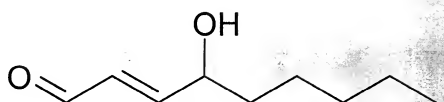
Cell Protection & Cancer Therapy

- **MarrowShield** - Single agent prophylactic for chemo-induced anemia, neutropenia, and thrombocytopenia therapy; (also military applications)
- **Cancer Therapy** – novel 'set-up & knock-down' treatment with PI3K inhibitors [i.e. temporarily expose to PTENi to cause cancer cells to be highly addicted to PI3K pathway interruption]
- **Cardiovascular** – a)protecting needed cells from ischemia/reperfusion injury; b) stimulate angiogenesis in diseased tissue

But where are PTEN INHIBITORS?

"Specific Inhibition of PTEN Expression Reverses Hyperglycemia in Diabetic Mice"; Butler et al, Diabetes 51:1028-1034, 2002

"4-Hydroxynonenal inhibits PTEN phosphatase in vitro"; Salsman et al, Proceedings of the AACR, Vol. 44, March 2003 abstract number 3470

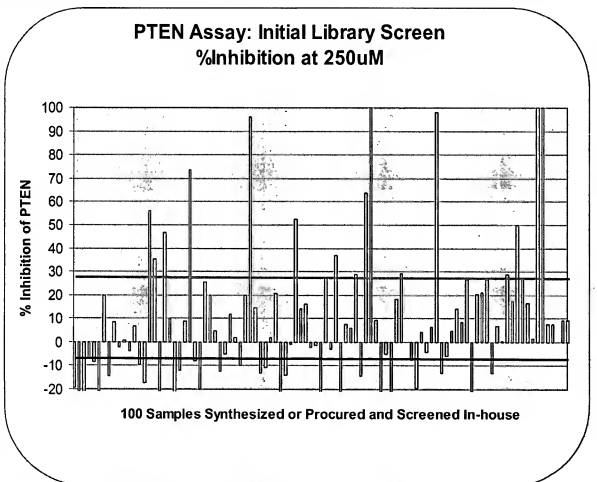


4-Hydroxynonenal HNE

"Bisperoxovanadium compounds are potent PTEN inhibitors" Schmid et al (Woscholski) FEBS Letters 566 (2004)35-38

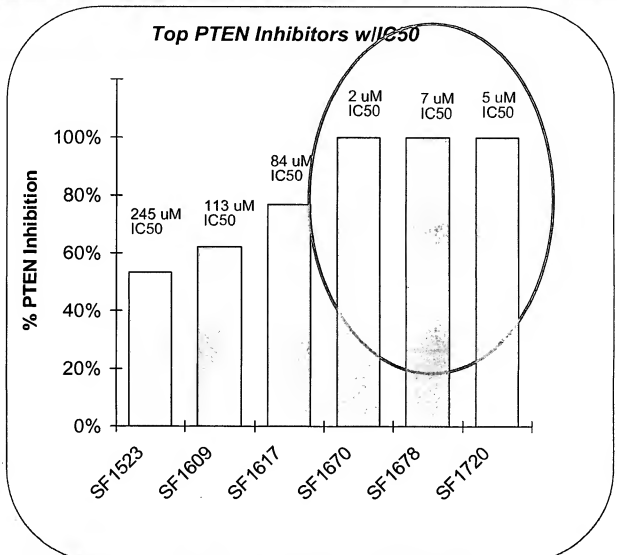
PTEN Program – Semafore's Discovery Process

Semafore has developed a robust in-house ***PTEN*** bioassay screening program

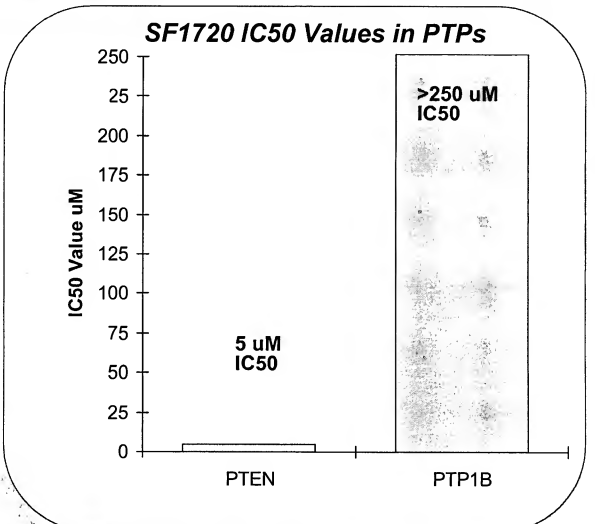


1. **In Silico –**
   a. Exclusive
   b. 9 million screened
   c. Top 3000 selected
   d. Library of 100
2. **In vitro – Semafore Bioassay Group**
   a. 250 uM in Level 1
   b. PIP3 as substrate (PLV)
3. **40% inhibition were confirmed and rerun**
4. **Enhanced Activity Noted (agonist activity)**
5. **IC50s determined**

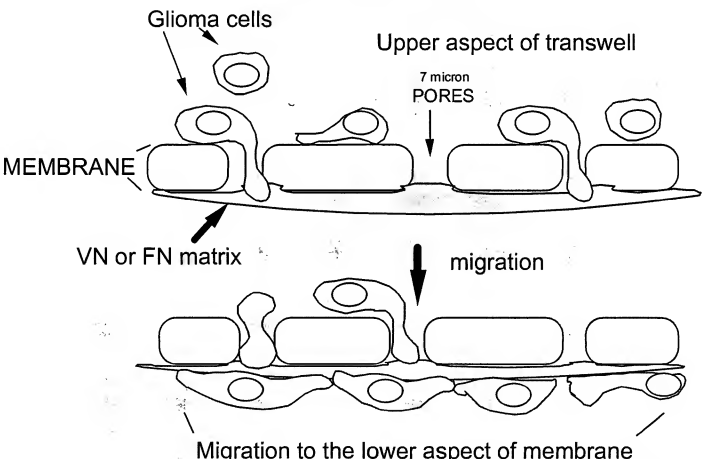
Proof Of Concept — First Known PTEN Inhibitor (Potency)



Selectivity – SF1720 (vs PTP1B—diabetes target phosphatase)

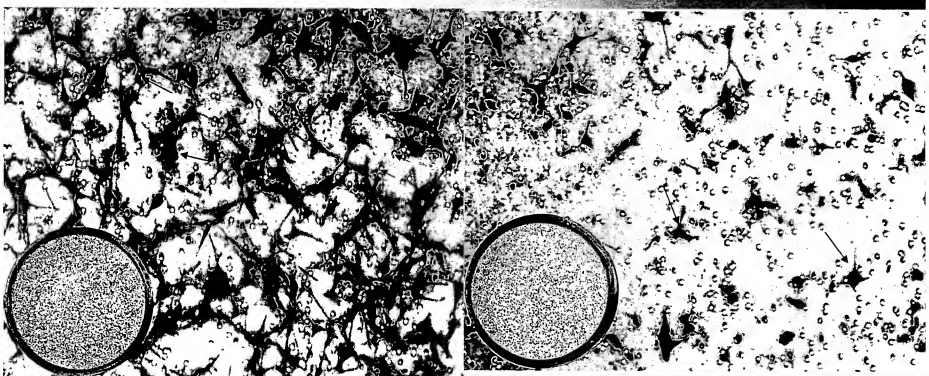


HAPTOTAXIS ASSAY



PTEN Contol of Migration

U87MG Migration on VN ($\alpha v\beta 3$)

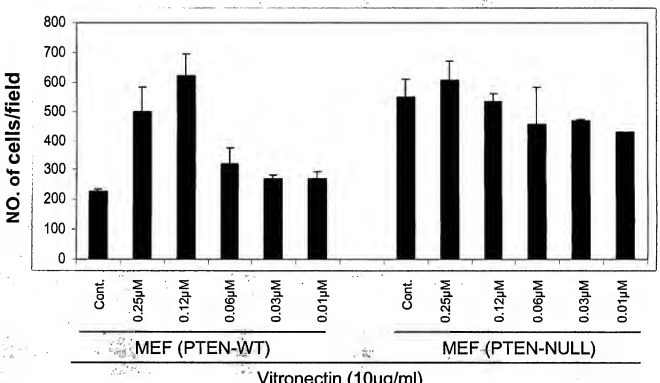


PTEN / NULL

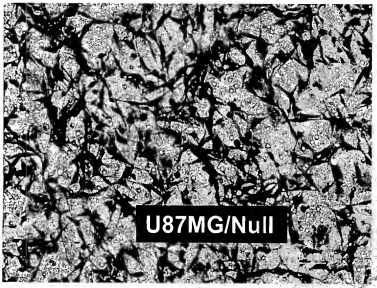
PTEN / WT

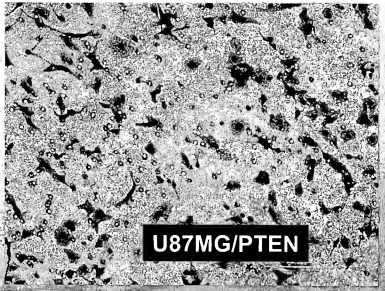
SEMAFORE PHARMACEUTICALS INC.

Role of PTEN inhibitor on integrin directed migration (mouse embryonic fibroblasts)

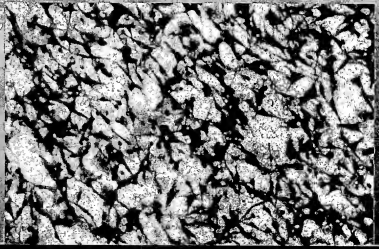


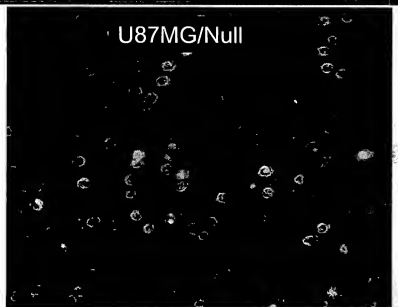
Migration Assay





Demonstrates Cellular Effects of PTEN Inhibitor (SF1670) Mimic PTEN Null Cells



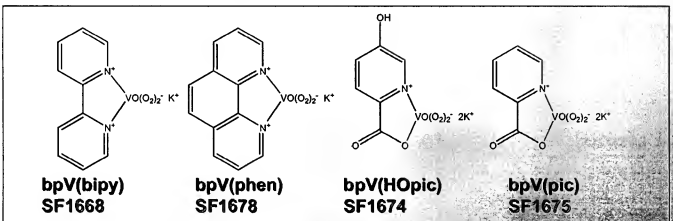


Merge

PTEN and PTP1B Activity of Selected Vanadate Compounds

Imperial College[1] and Semafore Pharmaceuticals Assay Results

[1] Schmid,A.C; Byrne, R.D.; Vilar, R.; Woscholski,R. Bisperoxovanadium compounds are potent PTEN inhibitors, *FEBS* **2004**, *566*, 35-38.



bpV(bipy) **SF1668**

bpV(phen) **SF1678**

bpV(HOpic) **SF1674**

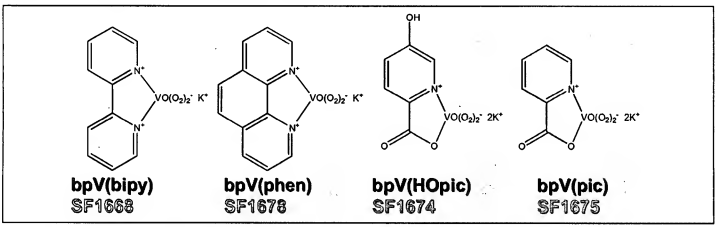
bpV(pic) **SF1675**

Compound		Imperial College[1]		
		PTEN	PTP1B	Ratio (PTEN : PTP1B 1mM pNPP)
bpV(bipy)	**SF1668**	18nM ±0.8	*164nM ±22.6*	1:9
bpV(phen)	**SF1678**	38nM ±2.4	*920nM ±46.2*	1:24
bpV(Hopic)	**SF1674**	14nM ±2.3	***25.3uM*** ±2.9	1:1807
bpV(pic)	**SF1675**	31nM ±1.7	***60.1uM*** ±6.8	1:1968
SUBSTRATE		PtdIns(3,4,5)P3	pNPP	

PTEN and PTP1B Activity of Selected Vanadate Compounds

Imperial College[1] and Semafore Pharmaceuticals Assay Results

[1] Schmid,A.C; Byrne, R.D.; Vilar, R.; Woscholski,R. Bisperoxovanadium compounds are potent PTEN inhibitors, *FEBS* **2004**, *566*, 35-38.

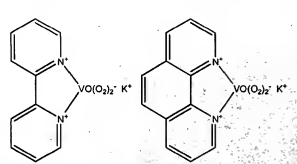


Compound		Semafore Pharmaceuticals			
		PTEN	PTP1B	PTP1B	Ratio (PTEN : PTP1B (pNPP))
bpV(bipy)	**SF1668**	276.3nM ±36.6	*213.7nM ±27.1*	*103nM*	1:1
bpV(phen)	**SF1678**	356.6nM ±91.4	*97.50nM ±19.8*	*83.98nM*	0.3:1
bpV(Hopic)	**SF1674**	91.1nM ± 6.4	*79.5nM ±22.1*	*45nM*	1:1
bpV(pic)	**SF1675**	111.2nM ±6.4	*118.4nM ±6.9*	*82nM*	1:1
SUBSTRATE		PtdIns(3,4,5)P3	pNPP	GluTyr	

PTEN and PTP1B Activity of Selected Vanadate Compounds

Imperial College[1] and Semafore Pharmaceuticals Assay Results

[1] Schmid,A.C; Byrne, R.D.; Vilar, R.; Woscholski,R. Bisperoxovanadium compounds are potent PTEN inhibitors, *FEBS* **2004**, *566*, 35-38.

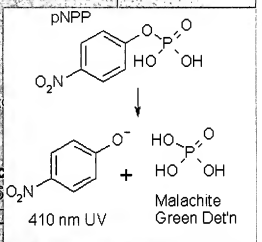


bpV(bipy) **SF1668**

bpV(phen) **SF1678**

bpV(HOpic) **SF1674**

bpV(pic **SF1675**

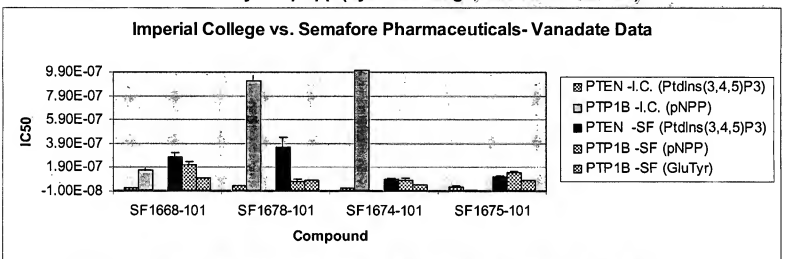


Compound		Semafore Pharmaceuticals			
		PTEN	PTP1B	PTP1B	Ratio (PTEN : PTP1B (pNPP))
bpV(bipy)	**SF1668**	276.3nM ±36.6	*213.7nM ±27.1*	*103nM*	1:1
bpV(phen)	**SF1678**	356.6nM ±91.4	*97.50nM ±19.8*	*83.98nM*	0.3:1
bpV(Hopic)	**SF1674**	91.1nM ± 6.4	*79.5nM ±22.1*	*45nM*	1:1
bpV(pic)	**SF1675**	111.2nM ±6.4	*118.4nM ±6.9*	*82nM*	1:1
SUBSTRATE		PtdIns(3,4,5)P3	pNPP	GluTyr	

PTEN and PTP1B Activity of Selected Vanadate Compounds

Imperial College[1] and Semafore Pharmaceuticals Assay Results

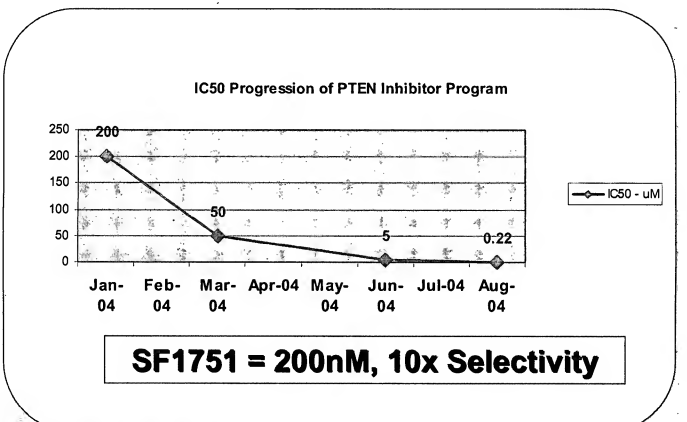
- **PTEN inhibitors have triple digit nanomolar inhibition, comparable to our SF compounds**
- **PTP1B inhibitors have triple digit nanomolar inhibition (no selectivity)**
- **PTP1B substrate- GluTyr vs pNpp (dynamic range, 410nM vs. 620 nM)**



Compound		Imperial College[1]	Semafore Pharmaceuticals
		Ratio (PTEN : PTP1B (pNPP))	
bpV(bipy)	SF1668	1:9	1:1
bpV(phen)	SF1678	1:24	0.3:1
bpV(Hopic)	SF1674	1:1807	1:1
bpV(pic)	SF1675	1:1968	1:1

PTEN Inhibitor Optimization – NanoMolar, Selective

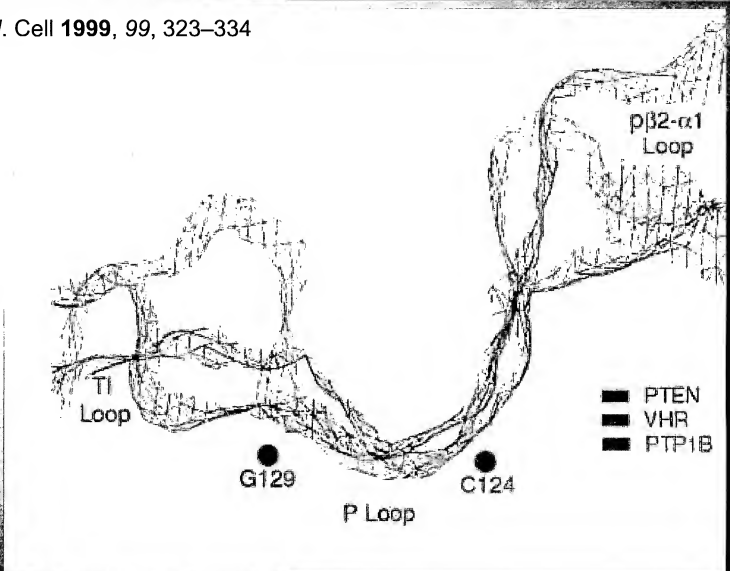
Semafore has optimized PTEN inhibitors from >200uM to less than 300 nM, with increasing selectivity vs. other phosphatases.



SF1751 = 200nM, 10x Selectivity

Selective PTEN Inhibitors-- Focus on Pocket Size and Shape

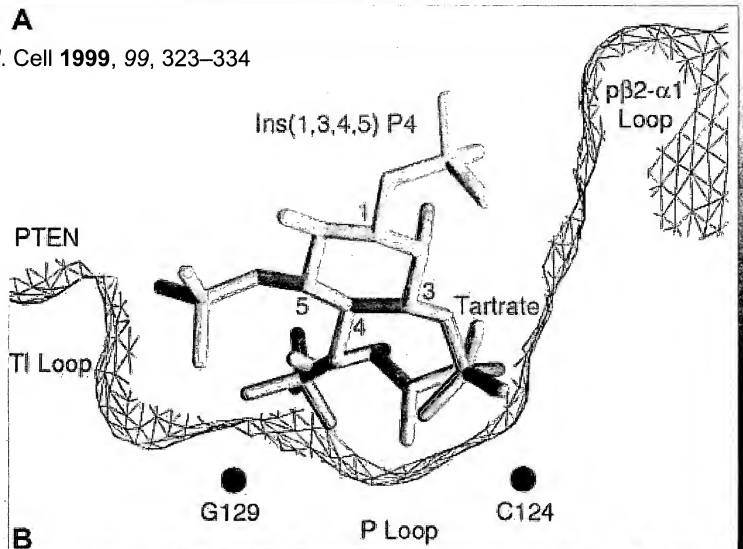
Lee *et al*. Cell **1999**, *99*, 323–334



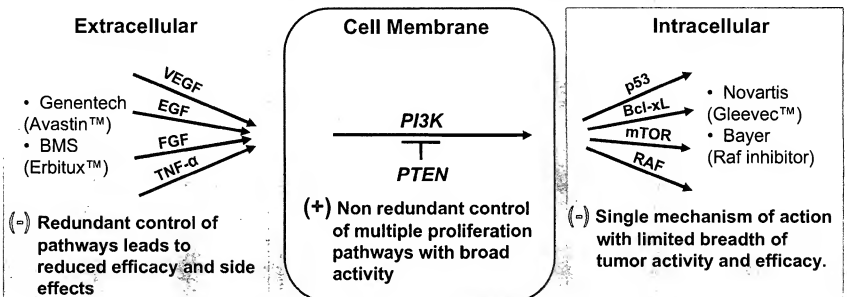
Selective PTEN Inhibitors-- Focus on Pocket Size and Shape

A

Lee *et al*. Cell **1999**, *99*, 323–334



SUMMARY



■Semafore Therapeutic Platforms

- ■*PI3K Inhibition—SF1126 PRECLINICAL*
- ■*PTEN Inhibition—Potency good; refining selectivity*

This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.
As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.